PROFILE OF PATIENTS WITH THROMBOSIS EVALUATED IN A TERTIARY CARE CENTER

DISSERTATION

SUBMITTED FOR

M.D. IN PATHOLOGY

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PATHOLOGY

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TAMILNADU, INDIA

FEBRUARY – 2009.

CERTIFICATE

This is to certify that the dissertation work entitled **"PROFILE OF PATIENTS WITH THROMBOSIS EVALUATED IN A TERTIARY CARE CENTER"** submitted by Dr. Aysha Ali is work done by her during the period of study in this department from June 2006 to February 2009. This work was done under the guidance of **Dr. Prasanna N. Kumar,** Professor, Department of Pathology PSG IMS & R.

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ACKNOWLEDGEMENT

It gives me immense pleasure to express my deep gratitude and heartfelt thanks to my respected teacher and guide Dr.Prasanna N. Kumar, Professor, Department of Pathology, PSG IMS & R for her invaluable guidance, immense patience and timey advice.

I express my sincere thanks to Dr. Alamelu Jayaraman, Professor and Head of the Department of Pathology, PSG IMS & R for her suggestions, encouragement and moral support.

I express my humble thanks to all faculty members of the department of Pathology, PSG IMS & R whom I have had the privilege to work with, especially Professor Dr. V.Nirmala, Associate Professors Dr. S. Shanthakumari, Dr. T.M.Subba Rao and Dr. Vanitha.S, Assistant Professors Dr.Suma. B.Pillai, Dr. M. Kanmani, Dr. Uma Maheswari,Dr.Rajesh and Dr. Divya Naik.

I am also very thankful to Dr. Ram Ganesh and Dr. Rajeswari who have been a source of strength in my research pursuit.

I am extremely greatful to Mrs. Asha.K and other Technical Staff of Clinical Pathology Section for their kind cooperation.

I am obliged to all patients who contributed to my study and findings.

CONTENTS

INTRODUCTION

A delicate balance exists between fibrin formation and fibrinolysis in vivo. Reduced blood flow, changes in the vessel wall and changes in blood composition result in disturbance of this balance, which favors fibrin formation and ultimately lead to formation of occlusive thrombi.

Venous and arterial thrombosis are common diseases with an estimated incidence of 1 in 1000 individuals per year[50] .Despite the growing insight in the pathogenesis of thrombophilia, the cause of many thrombotic episodes remain unknown. Although the causes are not always identified, several risk factors have been studied and it was observed that hypercoagulability is one of the triggers that alters the hemostasis. Hypercoagulability may occur due to defective naturally occurring anticoagulant mechanisms or due to heightened levels of procoagulant factors. Several risk factors have been identified like increased levels of coagulation factors-factor VIII, IX, XI, II and fibrinogen which are cumulatively explored by activated partial thromboplastin time used over the last 50 years as a standard screening test in clinical laboratories throughout the world. Several groups have identified elevated levels of factor VIII as an independent risk.

Antiphospholipid antibody syndrome is a clinical entity with a strong tendency for thrombosis accompanied by high mortality and morbidity. It is characterized by persistently elevated levels of antibodies directed against anionic phospholipids. As a result of antibodies homeostatic regulation of blood coagulation is altered. The diagnosis is made when arterial or venous thrombosis or recurrent pregnancy failure occurs in a patient with a laboratory test result persistently positive for an antiphospholipid antibody.

Paroxysmal nocturnal hemoglobinuria is a disease due to clonal disorder of the hematopoietic stem cell. This disorder is characterized by increased sensitivity to complement mediated lysis which affects all blood cells including platelets, which results in an increased sensitivity to venous thrombosis.

Sickle cell disease is an inherited disorder characterized by the presence of sickle hemoglobin which results from substitution of glutamic acid by valine at the sixth position of beta globin chain. Nearly every component of hemostasis including platelet function, procoagulant, anticoagulant and fibrinolytic system is altered and is associated with an increased risk of venous and arterial thrombosis.

This study is aimed at identifying the acquired risk factors of thrombosis like antiphospholipid antibody syndrome, elevated factor VIII level,

shortened activated partial thromboplastin time , paroxysymal nocturnal hemoglobinuria and sickle cell disease, so that the patients at risk can be identified and prevented from the occurrence of thrombosis. Previous studies have been done mostly in the European population, so this study also aims in identifying the risk factors involved in the pathogenesis of thrombosis in Indian population. It is also aimed in evaluating the investigations of antiphospholipid antibodies.

AIMS AND OBJECTIVES

1. To examine the risk factors involved in the pathogenesis of thrombosis in the Indian population.
2. To investigate the significance of these risk factors in the incidence of venous and arterial thombosis.
3. To evaluate investigations for the diagnosis of antiphospholipid antibodies.
4. To evaluate the significance of two different antiphospholipid antibodies – lupus anticoagulant and anticardiolipin antibodies in the pathogenesis of thrombotic disorders.

MATERIALS AND METHODS

Blood samples were collected from patients presenting with the clinical picture of arterial or venous thrombosis to the Departments of Cardiology, Neurology, Orthopaedics, Obstetrics & Gynaecology and Dermatology. 2.7 ml of blood was collected in tubes containing 0.3 ml of 3.2% trisodium citrate (ratio of 9:1); 2 ml in plain tubes for serum and 3 ml in tubes containing 1 ml of EDTA (Ethylene Diamine Tetra Acetic acid). The following investigations were done on the blood samples:

Complete blood count

Sickling test

Ham's acidified serum test

Sucrose lysis test

Screening tests for coagulation

Prothrombin time

Activated partial thromboplastin time

Lupus anticoagulant assay (dRVVT based)

Factor VIII:c (one-stage aPTT based) assay

Anticardiolipin anibody

20 control samples were run for lupus anticoagulant, antiphospholipid antibody and APTT and all the values were within normal limits. The statistical analyses of the results were done using a chi-square.

1. COMPLETE BLOOD COUNT

This was done using the patient's EDTA blood sample on a Beckman Coulter which is a 26 parameter fully automated hematology analyzer with five part leukocyte differential count.

2. SICKLING TEST

This was done using the Sicklevue test kit manufactured by Tulip diagnostics.

PRINCIPLE:

This test is based on the solubility difference between HbS and HbA in concentrated buffer solution. Red blood cells under test are lysed by a powerful hemolytic agent and the released hemoglobin is then reduced by sodium dithionate in a concentrated phosphate buffer. HbS precipitates in the presence of sodium dithionate causing turbidity of the reaction mixture, which is easily

visualized. Under the same conditions, HbA as well as most other hemoglobins are soluble, they remain in solution resulting in a clear suspension.

MATERIALS NEEDED:

Patients and control EDTA anticoagulated blood, solubility test reagent (concentrated phosphate buffer solution containing red cell lysing agent), empty reaction tubes, 2 ml pipettes, test tube, stopwatch, laboratory centrifuge.

METHOD:

All reagents and samples are brought to room temperature before use. The reaction tubes are labeled appropriately and set on a test tube rack. 100 µl of EDTA anticoagulated blood sample from the patient is added to 2 ml of the solubility test reagent (concentrated phosphate buffer solution containing red cell lysing agent). This is mixed for 10 seconds and allowed to stand for 10 minutes. The tubes are then centrifuged at 1200 g for 5 minutes. The pattern formed in the reaction tubes is observed**.** A control is also run simultaneously by using a normal subject's sample.

INTERPRETATION

In normal people (Hb-AA) the lower layer will be clear and dark red in colour and upper layer will have a grey precipitate.

In sickle cell anemia (Hb-SS), the lower layer will be colourless and upper layer will have red precipitate.

In sickle cell trait (Hb-AS), the lower layer will be clear and light red to pink in colour while the upper layer shows red precipitate.

3. SUCROSE LYSIS TEST

PRINCIPLE:

The principle of this test is that red cells absorb complement components from serum at low ionic concentrations. Red cells from cases of paroxysmal nocturnal hemoglobinuria undergo lysis because of their greater sensitivity to lysis**.**

MATERIALS REQUIRED:

50% Suspension of patient's red cells, isoosmotic solution of sucrose (92.4 g/l), fresh normal ABO compatible serum, normal saline, spectrophotometer.

PROCEDURE:

An isoosmotic solution of sucrose is required (92.4 g/l). This can be stored at 4 degree Celsius upto 2-3 weeks.

Two tubes are set up, one containing 0.05 ml of fresh normal ABO compatible serum diluted in 0.85 ml of sucrose solution and the other containing 0.05 ml of fresh normal serum diluted in 0.85 ml of saline. To each tube 0.1 ml of 50% suspension of washed red cells is added. The tubes are incubated at 37° Celsius for 30 minutes, centrifuged and observed for lysis. The quantum of lysis is measured using a spectrophotometer.

INTERPRETATION:

In paroxysmal nocturnal hemoglobinuria, 10-80% lysis is observed. <10% lysis may be seen in certain leukemias and myelosclerosis.[10]

4. HAM'S ACIDIFIED SERUM TEST

PRINCIPLE:

Patient's red cells obtained from EDTA or defibrinated blood are exposed at body temperature to the action of fresh normal serum acidified to optimum pH for lysis (pH 6.5-7.0).

METHOD:

0.5 ml samples of fresh normal AB group serum or serum that is ABO compatible with the patient's blood are delivered into six test tubes. Two of the tubes are placed at 56° C for 10-30 minutes in order to inactivate complement.

The other two pairs are kept at room temperature and 0.05 ml of 0.2 ml/l hydrochloric acid is added. Similar volumes of acid are added to the inactivated serum samples. All the tubes are incubated at 37°C. 50% suspensions of washed patient's and control red cells are added to the tubes containing unacidified fresh serum, acidified fresh serum and acidified inactivated serum. These tubes are then incubated at 37° C for 1 hour. The tubes are centrifuged after incubation.

INTERPRETATION:

In a patient with paroxysmal nocturnal hemoglobinuria, lysis is seen in the tube containing acidified serum. There is no lysis in the tube containing non-acidified or heat inactivated serum. The control cells do not undergo lysis in any of the three test tubes. The percentage of lysis may be measured using a spectrophotometer at a wavelength of 540 nm.

5. PLASMA CLOTTING TESTS

a. Equipment – STAGO coagulation analyzer, water bath, centrifuge, freezer, pipettes, glassware and disposable plastic tubes, timers.

b. Reagents include tissue thromboplastin, APTT reagent, calcium chloride, anticoagulants, pooled plasma, reference plasma and factor deficient plasma.

c. Samples - Blood from both controls and patients was collected by clean venipuncture, using a 21 guage needle, into a tube containing 3.2% trisodium citrate in a ratio of nine parts blood to one part citrate. The samples were centrifuged at 1500g for 15 minutes to prepare platelet poor plasma (PPP). For the lupus anticoagulant and anticardiolipin tests which were not done immediately the plasma was removed into plastic tubes, centrifuged a second time at 1500 g for 15 minutes and aliquots of this platelet–depleted plasma were stored at -80° C until testing. The platelet count of the platelet depleted plasma was $< 5 \text{ X } 10^9/ \text{ L}$.

d. Anticoagulants - 3.2% sodium citrate in a ratio of 1 part anticoagulant to 9 parts of blood for clotting tests.

A. PROTHROMBIN TIME

PRINCIPLE:

The prothrombin time is the time taken for citrated plasma to clot on the addition of tissue thromboplastin and calcium chloride. It measures the extrinsic and common pathways i.e. Factors I, II, V, VII and X.

The source of tissue thromboplastin varies and may be from human or animal brain, placental extract or recombinant. In this test freeze dried thromboplastin derived from rabbit cerebral tissues was used. The

International Sensitivity Index of the thromboplastin reagent that was used for this study was 1.21**.** The reagent comes mixed with calcium chloride.

METHOD:

The test is performed at 37^0C, mixing one part of plasma with two parts of the reagent calcium chloride mixture and timing the plasma clot formation. A normal control is done each time. Every test is run in duplicate. The normal PT by this technique is 11 – 16 seconds. A greater than 2 seconds difference from the control is considered significant.

B. ACTIVATED PARTIAL THROMBOPLASTIN TIME

PRINCIPLE:

Activated partial thromboplastin time is the time taken for citrated plasma to clot on the addition of a surface activator, phospholipid and calcium chloride. APTT measures the Intrinsic and Common pathways. It measures Factors I, II, V, VIII, IX, X, XI, and XII. It does not measure FVII and FXIII activity.

The activators used are kaolin, silica, celite, actin or ellagic acid**.** The activator used in this study is silica. The partial thromboplastin is phospholipids.

Lupus anticoagulant exerts an inhibitory effect on phospholipids which are required in the clotting pathways, Hence the presence of lupus anticoagulants prolongs clotting time.

METHOD:

Lupus sensitive APTT reagent which is commercially available as a freeze dried preparation containing cephalin and a particulate activator (silica) in a buffered medium is used.

One part of plasma is mixed with one part of activator and phospholipid, incubated at 37^0C and one part of calcium chloride is added and the time to clot is noted.

INTERPRETATION:

The normal APTT by this method is 28 - 35 seconds. A greater than 5 seconds difference from the control is considered abnormal. A shorter than normal APTT indicates an activated sample or a hypercoagulable state.

CORRECTION STUDIES:

When the APTT is prolonged, correction studies are done to differentiate whether the prolongation is due to an inhibitor or a factor deficiency. This is done by mixing equal volumes of the test plasma and control pool plasma and repeating the test. When inhibitors are present the prolonged timing will not correct.

6. LUPUS ANTICOAGULANT ASSAY (dRVVT based):

PRINCIPLE:

Russell's viper venom activates factor X in the presence of phospholipid and calcium ions. Lupus anticoagulant prolongs the clotting time by binding to phospholipid and preventing the action of Russell's viper venom.

MATERIALS REQUIRED:

Platelet poor plasma- from patient and control Glyoxaline buffer, Russell's viper venom (lyophilized), calcium chloride 0.025 mol/l, phospholipid.

METHOD:

This test is performed at 37°C, mixing one part of platelet poor plasma to one part of a lyophilized preparation of Russell's viper venom and phospholipids and one part of 0.025M calcium chloride and timing the plasma clot formation. This is the 'screen time' which should be normally 28-45 seconds.

When the screen time is more than 45 seconds, the test is repeated using the Lupus Anticoagulant Confirm kit, which contains excess of phospholipids.

INTERPRETATION:

Ratios of mean screen time/mean confirm time <1.0 is normal.

In this study, if the screen time and confirm time is more than 45 seconds and the ratio between screen and confirm is more than 1 , then it was labeled as lupus anticoagulant positive.

7. ANTICARDIOLIPIN ANTIBODY TEST:

PRINCIPLE:

Cardiolipin IgA, IgM, IgG kits are available for determination of autoantibodies directed against cardiolipin in human serum. The kits used are solid phase enzyme immunoassays. Microtitre wells are coated with cardiolipin and β2 –glycoprotein 1. The presence of cardiolipin and β2 –glycoprotein 1 complexes allows specific cardiolipin antibodies to bind to the solid phase.

MATERIALS REQUIRED:

Microtitre plate reader with 450 nm reading filter

Microplate washing device

Microtitre plates-12×8 well strips with breakaway microwells

Calibrators- containing diluted human serum and sodium azide as preservative

Conjugates-15ml IgG, 15ml IgM (anti-human immunoglobulins conjugated to horseradish peroxidases), TMB substrate-15 ml(stabilized hydrogen peroxide), stop solution- 1M hydrochloric acid,

Sample buffer –containing trisodium chloride, sodium azide, BSA, wash buffer containing tris, sodium chloride, tween, and sodium azide

METHOD:

Antigen precoated microplate wells are incubated with calibrators, controls and serum specimens. During the incubation, antibody present in the test sample binds to coated wells. The wells are then washed to remove unbound antibodies and horse radish peroxidase labeled anti-human Ig is incubated into the wells. Chromogen is added and autoantibodies are measured using a spectrophotometric plate reader using a 450 nm filter.

INTERPRETATION:

Calibration curves are made with the absorbance value at 450/630 nm on the Y axis and the cardiolipin class specific Ig on the X axis. The concentration of anticardiolipin class specific antibody is read from the calibration curve. Values > 15 MPL/ml for IgM and >15 GPL/ml for IgG [10] was labeled as positive

8. FACTOR VIII ASSAY:

PRINCIPLE:

The principle of the assay is based on the ability of reference plasma and the test plasma to correct the prolonged APTT of plasma deficient in the one factor that is being assayed – in this case Factor VIII. For the assay, reference

plasma with known content of all the factors and factor VIII deficient plasma with factor level less than 1% activity must be available.

MATERIALS REQUIRED:

STAGO coagulation analyzer, APTT reagent (STAGO CK PREST), Owren Koller buffer, calcium chloride 0.025 M, system control plasma- normal and abnormal, Factor VIII deficient plasma (Stago)

METHOD:

One part of test plasma is mixed with one part of factor VIII deficient plasma and one part of APTT reagent, incubated at 37^0C, one part of calcium chloride is added and the time taken to clot is noted.

Abnormal system controls with a Factor VIII level of 32 – 46% and normal system controls with Factor VIII level of 87-121% are subjected to the test simultaneously with patient's sample.

INTERPRETATION:

Factor VIII level is expressed as percentage of the activity of normal plasma.

Normal levels are 50- 150%[10]

REVIEW OF LITERATURE

Thrombosis may be defined as formation and propagation of blood clot within the vasculature[56]. When a blood vessel is damaged and normal endothelial cell barrier is disrupted platelets are recruited from the blood vessel to the site of damage to form an occlusive plug. This is by adhesion and aggregation mediated by von Willebrand factor and fibrinogen

After the formation of primary hemostatic plug, the coagulation system is triggered when factor VIII combines with tissue factor leading to step wise activation of a series of proenzymes to produce thrombin. Thrombin activates platelets leading to exposure of negatively charged phospholipids which leads to clotting factor assembly further helping in thrombin formation. Thrombin clots fibrinogen to fibrin which is then crosslinked and anchored into place by process of clot retraction. Thus, clot formation is mediated by adhesive proteins and their receptors and proenzymes and their activators. This process is regulated by antithrombin III, thrombomodulin and protein C, protein S system which inactivate accelerator of thrombin formation (Factor Va and factor VIIIa). Subsequently clot is lysed by plasmin formation by the fibrinolytic system[17].

There is a balance between fibrin formation and fibrinolysis. Decreased blood flow, changes in vessel wall and hypercoagulability may lead

to disturbance of this balance which favors fibrin formation and formation of occlusive thrombi[17]. Venous thrombi occur as a result of clot formation in a vein due to decreased blood flow. Arterial thrombosis occurs due to formation of platelet aggregates at the site of vessel wall injury[9].

The causes of arterial and venous thrombosis can be hereditary or acquired. The hereditary causes are antithrombin III deficiency, protein C deficiency, protein S deficiency, dysfibrinogenemia, elevated lipoprotein levels, elevated factor VIII levels etc. Acquired causes described are antiphospholipid antibody syndrome, paroxysmal nocturnal hemoglobinuria, malignancies, myeloproliferative disorders, autoimmune hemolytic anemia, nephrotic syndrome, pregnancy, immobiliztion etc [17,23,32,44].

When approaching a patient with a thrombophilic disorder, it will be useful to differentiate between patients with acquired and hereditary hypercoagulable states. Hereditary disorders are often attributed to a single mutation of protein in a critical anticoagulant pathway, whereas acquired hypercoagulable states consist of a heterogenous group of disorders that are associated with an increased tendency towards thrombotic complications [32, 56]. A complete history and physical examination is necessary while evaluating patients with thrombosis[4,57].

An individual can be labeled strongly or weakly thrombophilic based on history. A strongly thrombophilic patient will have three important features[5]-

1. First thromboembolic event occurring prior to the age of 50
2. History of recurrent thrombotic episodes

3 A first degree relative with a documented venous thromboembolic event prior to age of 50.

A strongly positive individual should have full laboratory investigation for thrombophilia like-factor V, homocysteine antiphospholipid antibody, protein C, protein S and a weakly positive individual should be investigated for antiphospholipid antibody, homocysteine, factor V[5, 6, 56].

Extensive tests are not cost effective for all patients who present with a thromboembolic event. For patients presenting with venous thrombosis, the criteria for undergoing a prothrombotic work up will be one or more of the following: recurrent thrombosis, family history of thrombosis, thrombosis at young age, thrombosis in locations other than deep veins of the leg. [5, 6]

For patients with arterial thromboembolism, the indications for a complete thrombotic workup will be one or more of the following: young age,

multiple thrombotic events, thrombosis in the absence of obvious atherosclerosis and when the patient has both arterial and venous thrombosis[5]

ANTIPHOSPHOLIPID ANTIBODY SYNDROME

Antiphospholipid antibodies are a family of autoantibodies that are directed against antigens that are composed of negatively charged phospholipids, phospholipid binding protein or both[7,23]. Clinically important antiphospholipid antibodies are lupus anticoagulant and anticardiolipin antibody[32]. Antiphospholipid antibodies have been associated with a number of disorders that include arterial and venous thromboembolism, thrombocytopenia, recurrent pregnancy loss, neurologic diseases etc[5,23].

Antiphospholipid antibodies require the presence of plasma phospholipid binding protein β2 glycoprotein 1, which is an apolipoprotein which binds avidly negatively charged phospholipids and is involved in a variety of platelet activation and coagulation [7,4,17,44]. The possible mechanism of thrombosis in antiphospholipid antibody syndrome could be a result of vascular or cellular injury[17, 44]. It is stated that it could probably be due to the antibodies blocking antithrombotic mechanism like decreased conversion of protein C to activated protein C, decreased destruction of factor Vc and VIIIa by activated protein C, low plasma protein S[6, 7]. It can also be due to increased platelet reactivity due to either decreased prostacyclin synthesis by endothelial

cells as a result of antiphospholipid antibody inhibhition of phospholipase A2 or due to antibody induced platelet activation[4,7,23,44]. Other proposed mechanisms are activation of complement[7], impaired fibrinolysis due to elevated plasminogen inhibhitor, and activation of coagulation by increased tissue factor synthesis by endothelial cells[5].

Most of the patients with autoimmune or rheumatic disease have antiphospholipid antibodies. Common autoimmune diseases associated with antiphospholipid antibodies are-SLE, Sjogren syndrome, rheumatoid arthritis, autoimmune thrombocytopenic purpura and autoimmune hemolytic anemia[7].

The antiphospholipid antibody syndrome may be divided into two main categories[5,7,44]. Primary -when it occurs in patients without any clinical evidence of other autoimmune disease. It is called secondary when it is associated with other autoimmune diseases like SLE[44]. Bick[5] et al describes an entity called antiphospholipid thrombosis syndrome, where lupus anticoagulant, anticardiolipin antibodies as well as recently recognized subgroups of antiphospholipid antibodies like antibodies against beta-2 – glycoprotein, antibodies to phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl glycerol , phosphatidyl inositol and anti-annexin V are included. He subcategorises antiphospholipid thrombosis syndrome into three groups according to the type of antibody involved-the LA thrombosis syndrome, the

ACLA thrombosis syndrome and thrombosis associated with subgroups of antibodies.

In 1952 Conley and Hartmann described a coagulation disorder in two patients with SLE [5.] The patients exhibhited anticoagulant activity by in vitro testing, which was manifested by a prolonged clotting time and prothrombin time. There also have been patients with systemic lupus eythematosus with phospholipid dependant inhibhitors of coagulation[17,44] Later in 1963, the term lupus anticoagulant was proposed for these antibodies, based on their prevelance in SLE[17.]Approximately 10% patients with SLE harbor lupus anticoagulant[4,5,6,25]. Lupus anticoagulant is seen in malignancies, lymphoproliferative disorders, viral infections and also seen in association with drugs like - chlorpromazine, procainamide, hydralazine, cocaine and dilantin[5]. Most commonly lupus anticoagulant develops in an otherwise healthy individual[5]. Patients with lupus anticoagulant are at more risk with thrombosis related events than anticardiolipin antibodies[38]. Most commonly it is associated with deep vein thrombosis[7,25,38]. It is also associated with recurrent miscarriage, neuropsychiatric disorders, renal vascular thrombosis, thrombosis of dermal vessels[4,5,7]. Primary LA is more common than secondary LA[5]. They usually have venous thrombosis and pulmonary emboli[5,15]. A wide variety of veins are involved including systemic veins like mesenteric, renal, hepatic etc. Arterial thrombosis is also observed in some patients with primary LA syndrome[5,6]. But it is less common than in patients with secondary LA syndrome[5]. Arteries

commonly involved include cerebral, coronary, carotid, aorta and arteries of extremities[4,5]. However, even in secondary LA syndrome, venous thrombosis is much more common than arterial thrombosis[4,6,7].

Purified LA inhibits calcium dependent binding of prothrombin and factor Xa to phospholipids, therefore inhibiting the activity of phospholipid complex which is required for the conversion of prothrombin to thrombin[6]. Therefore an abnormality often exists in phospholipid dependant coagulation reactions, including prothrombin time, activated partial thromboplastin time and Russell's viper venom test, because the LA is not directed against a specific factor, but to phospholipids[5,16,30,31,50]. The inhibitor doesn't usually exert inhibitory effect after prolonged incubation. This property can be used to differentiate between lupus inhibitors and other inhibitors of clotting factors. Approximately 15- 25 % of cases can be time dependant so this test is not definitive. Multiple assays are in use in many laboratories. Sensitivity of activated partial thromboplastin time to the presence of lupus anticoagulant is highly dependant on the reagent used[6,25,27,30,31]. Many patients with LA have normal APTT[5,6]. So this test is not an appropriate screening test for LA.

The screening tests should be sensitive to LA. However the basis of sensitivity is still not completely understood. A common concept is that the amount of phospholipid in the test system is a critical determinant of sensitivity[6,30]. Tests like kaolin clotting time, dRVVT, dilute APTT, dilute

prothrombin time has increased sensitivity because they have reduced amount of phospholipids[5,6,30].The hemostasis committee of the "socie francaise de biologie Clinique" found that some weak to moderate LA's were detected with one but not another assay[5,6]. So they suggested that multiple types of sensitive assay to enhance the overall sensitivity. Brant et al suggest more than one type of assay should be done for screening[6]. Screening assays for lupus anticoagulants suggested by Brant et al is as follows - activated partial thromboplastin time, dilute activated partial thromboplastin time, dilute Russell viper venom test, dilute prothrombin time, kaolin clotting time, silica clotting time[6]. All of these are phospholipid dependant clotting tests[5,6,30].

The scientific subcommittee criteria for diagnosis of lupus anticoagulants are the following[6]:

1. Prolongation of at least one phospholipid dependant clotting tests.
2. Evidence of inhibitory activity shown by the effect of patient plasma on pooled normal plasma.
3. Evidence that the inhibitory activity is dependant on phospholipid.

LAs must be carefully distinguished from other coagulopathies that may give similar laboratory results or may concurrently occur with LAs.

Specific factor assays and clinical histories maybe helpful in identifying LAs from other diseases[5,6].

Anticardiolipin antibodies are associated with thrombosis and thromboembolism of arterial and venous systems, recurrent miscarriages and thrombocytopenia[5,25,44]. They are associated with many types of venous thrombotic problems including deep venous thrombosis of upper and lower extremities, pulmonary embolus, intracranial veins, inferior and superior venacava, hepatic veins, portal vein, renal vein and retinal veins[5,17,56]. Coronary arteries, carotid arteries, cerebral arteries, subclavian or axillary artery, brachial arteries, mesenteric arteries are the sites of arterial thrombosis associated with anticardiolipin antibodies[17,56]. It is also associated with livido reticularis, transient ischemic attacks, small stroke syndrome, valvular heart diseases and acute myocardial infarction[5]. The obstetric complications associated with anticardiolipin antibodies are frequent abortion in first trimester due to placental thrombosis[20] or vasculitis, recurrent fetal loss in second and third trimester[20], also due to placental thrombosis or vasculitis, and maternal thrombocytopenia[5,7]. The primary phospholipid syndrome often is present in patients with a constellation of concomitant arterial occlusions, strokes, transient ischemic attacks leading to multiple infarct dementia, deep vein thrombosis associated with pulmonary embolization and resultant pulmonary hypertension, recurrent miscarriage[5,7,18,20,25]. Anticardiolipin antibodies have been reported in patients with HIV infection[7], however there is no correlation

between antiphospholipid antibody level and disease progression or incidence of thrombosis. Elevated anticardiolipin antibodies are seen in a number of acute infections like-varicella, rubella, Lyme disease, mycoplasma, certain drugs like procainamide, cocaine, phenytoin which may progress to a prothrombotic disorder sometimes[5,44].

The antiphospholipid thrombosis syndrome associated with anticardiolipin can be divided into 6 subgroups depending on the site of thrombus formation[5]. The detection of anticardiolipin antibody is by ELISA-36% have isolated IgG, 17% IgM, 14% IgA, 33% mixture of all[5,7]. So it is better to measure all three antibodies.

International consensus statement gives clinical and laboratory criteria for the diagnosis of antiphospholipid antibody syndrome[38,44].

Clinical criteria is as follows:[44]

1. One or more clinical episodes of arterial, venous or small vessel thrombosis occurring within any tissue or organ.

2. One or more unexplained deaths of morphologically normal fetuses at or after the tenth week of gestation; or one or more premature births of morphologically normal neonates at or before 34th week of gestation; or

three or more unexplained consecutive spontaneous abortion before tenth week of gestation.

Laboratory Criteria:

1. Anticardiolipin antibodies IgG or IgM at moderate or high levels in the blood on two or more occasions at least six weeks apart.

2. Lupus anticoagulant antibodies detected in blood on two or more occasions at least six weeks apart[7,44].

Therefore, the hallmark laboratory result which defines an antiphospholipid antibody syndrome is the presence of antiphospholipid antibody detected by phospholipid dependents tests of coagulation[44]. Steven et al suggests that in a suspected case of antiphospholipid antibody syndrome, anticardiolipin antibodies, anti beta -2 glycoprotein 1 antibodies, activated partial thromboplastin time, lupus anticoagulant by DRVVT, tests for syphilis (false positive) as well as a complete blood count should be done, complete blood count reveals thrombocytopenia and Coomb's positive hemolytic anemia[7].

ELEVATED FACTOR VIII LEVELS AND THROMBOSIS.

Elevated plasma factor VIII level is associated with recurrent venous as well as arterial thromboembolism. Several prospective studies in healthy individuals shows an association between elevated factor VIII:C levels and von Willebrand factor levels and the incidence of ischemic heart disease[33,39]. In a study by Leiden Thrombophilia it was observed that individuals with levels of factor VIII :C exceeding 150 IU/dl had a three fold increased risk compared with those with levels lesser than 150 IU/dL[33,39,42]. However, the cause of elevated factor VIII: C, which is an acute phase reactant is still not completely understood.

There is a genetic influence on the level of factor VIII: C. It has been suggested that a primary role of X linked genetic determinants on the basis of observation of positive correlation of factor VIII:C levels within groups of male pairs who had identical X alleles. von Willebrand factor and blood group are important determinants of factor VIII level in the plasma[12,33]. Non O blood group is associated with higher von Willebrand factor and factor VIII levels than in blood group O[33]. Individuals with AB blood group have highest von Willebrand factor levels. Blood group A, B and H oligosaccharide structures have been identified on vWF/factor VIII complex[33,39].

Since factor VIII:C is elevated in response to acute phase reaction[5,12,42]. It is difficult to determine whether increased factor VIII: C precedes thrombosis or whether it is a secondary reactive phenomenon. Kamphausein et al opines that elevated factor VIII levels in patients with thrombosis persist over time[39]. So they possibly are not caused by acute-phase reactions. O'Donell et al showed that only 50% of persistently elevated factor VIII levels were associated with high vWF:Ag levels, indicating that von Willebrand factor is not always responsible for high factor VIII plasma levels[37]. However some authors recommend that if a patient presenting with thrombophilia has elevated factor VIII levels, shortly after the thromboembolic event, the test should be repeated after two or three months[33,39].

The cause of elevated factor VIII:C levels in the plasma in a thromboembolic event is not properly understood. It was observed that factor VIII C, even several months after the acute thrombotic event were elevated[25,27]. Furthermore the correction for C-reactive protein and fibrinogen-well known acute phase proteins did not alter the observed odd ratios. So an acute phase response is ruled out. Consistent elevation of factor VIII C supports the notion that it is the intrinsic factor VIII:C levels which is responsible for the association with venous thromboembolism[12,37,42]. Another possibility is that high plasma concentration of factor VIII C is partially determined by genetic factors[39]. Kamphausein et al noted a high concordance of factor VIII levels between first degree relatives of patients with thrombosis with high factor VIII

levels. No variations in factor VIII or vWF gene that are associated with high factor VIII levels have been observed. High levels of factor VIII are likely to be expressions of "gain in function mutation"[42.]

Other determinants of plasma factor VIII levels are body mass index, high levels of blood glucose, insulin, fibrinogen and triglycerides. It is also observed that factor VIII levels increase with age[25], with an average rise of 5 to 6 IU /dl per decade. Exercise transiently induces a rise in factor VIII levels. This may probably be a result of adrenaline stimulation [12]. Sustained rise in the plasma factor VIII levels are seen during pregnancy, surgery, chronic inflammation, malignancy, liver disease, hyperthyroidism, intravascular hemolysis and renal disease[12,25,39].

In a study based on venous thrombosis by Leiden Thrombophilia , non O blood group, von Willebrand factor: Ag, and factor VIII:C were all associated with an increased risk for venous thrombosis by univariate analysis. In multivariate analysis factor VIII: C levels were consistently elevated in patients with thrombosis, but the effect of blood group and vWF:Ag on thrombosis were almost disappeared[12,22,27,49]. This suggests that factor VIII is an independent risk factor for venous thrombosis and that vWF and blood group are only risk factors in so far as they affect the factor VIII level. As mentioned earlier, factor VIII levels >150 IU /dl is at high risk for developing thrombosis[16,19,21]. Each increase in factor VIII level of 10 IU/dl is associated

with a 10% increase in the risk of a first thrombotic event.[12,39]. For recurrent disease this figure is 24%, showing that elevated factor VIII is an even stronger risk for recurrent disease[22]. In previous studies antigen method was used for factor VIII assay, but one stage clotting assay was also found to have equal results [22]. But Wells et al opines that these values do not identify patients at risk of recurrence [54]. He also suggests that age and gender should also be considered before the labs establish factor VIII levels that identify risk for venous thromboembolism [49],[54].

Determining patients with elevated levels of factor VIII that attribute to venous thromboembolism may be important for family risk evaluation. There are evidences that there is an independent genetic cause for persistently elevated factor VIII levels and a quantitative locus on chromosome 18 [54]. Genetic alterations that correlate with elevated levels of factor VIII were not identified, but heritability has been demonstrated [54]. Therefore determination of elevated factor VIII levels in patients with venous thromboembolism should lead to consideration of screening of first degree relatives [18 54].

Less commonly elevated factor VIII levels are seen in arterial thrombosis. The first reports on a possible association between factor VIII and coronary artery disease was in 1960"s [17].In the same period , non O blood and von Willebrand factor were identified as candidate risk factors for atherothrombotic disease [22,33,39]. Several studies have shown association

between elevated factor VIII levels and vWF levels with ischemic heart disease[33,39.]

Meade et al found that factor VIII remained associated with ischemic heart disease after adjustment of blood group, without taking vWF into account[33]. In the Hoorn study, high von Willebrand factor levels were associated with cardiovascular mortality independent of blood group in diabetic and non diabetic subjects [33]. When vWF and factor VIII are mutually adjusted for, neither of the two remained associated with coronary artery disease. Therefore, it is likely that factor VIII and vWF increase the risk of arterial thrombosis and this is independent of blood group[33]. Meade et al suggested that factor VIII is associated with ischemic heart disease, perhaps as a marker of vessel wall changes exerted by vWF antigen and factor VIII: C contributing to the degree of coagulability[33].

The ARIC study demonstrated strong association of factor VIII and vWF with risk factors for atherosclerosis such as hypertension, diabetes mellitus, body mass index and triglycerides. Some of these factors are known to be associated with perturbed endothelial and vascular inflammation. High shear forces, like those in stenosed vessles, increase vWF secretion by vascular endothelium and thus will stimulate platelet adhesion and aggregation at the site of damaged arterial walls, which may lead to thrombus formation[12,39]. This explains the elevation of factor VIII levels in stroke patients with presumed

large vessel disease[39]. High factor VIII levels may stimulate formation of thrombin and thus result in increased platelet activation and fibrin formation[39]. Past studies have found that there is low cardiovascular mortality in hemophilia patients. This also supports that elevated factor VIII levels are associated with arterial thrombosis[33,39,42].

SICKLE CELL DISEASE AND HYPERCOAGULABILITY

Sickle cell disease is an inherited disorder characterized by the presence of sickle hemoglobin, which results from the substitution of glutamic acid by valine at the sixth position of the beta globin chain. These patients will have hemolytic and vaso occlusive crisis, which is well known for years. Recently it was found that these patients are at risk for a variety of thrombotic complications[2]. Ischemic stroke due to occlusion of large vessel and superimposed thrombosis occurs commonly in patients with sickle cell anemia[14]. It has also been reported that it is associated with pulmonary embolism, deep vein thrombosis and several events of venous thrombosis [2].

Sickle cell disease affects several components of hemostasis like, platelet function and the procoagulant, anticoagulant and fibrinolytic system. Hence it is often called as a hypercoagulable state [2,17]. Red blood cells have phosphatidyl serine in inner monolayer of the cell membrane, and phospholipids in the outer monolayer in the plasma membrane. RBC

membrane asymmetry is maintained by the action of an ATP-dependant aminophospholipid translocase[45]. This is lost in sickle cell disease. Abnormal phosphatidyl serine exposure occurs as a result of sickling and unsickling which results in production of terminal spicules with exposed phophatidyl serine. This abnormal external exposure of phosphatidyl serine alters the hemostatic properties of RBC's which lead to thrombosis[2,17,45].

The Tissue factor-factor VIIa complex is the initiator of hemostasis[2]. In sickle cell disease abnormal expression of tissue factor by endothelial cells have been observed[2,45]. This expression is increased during pain crises[2]. It is found that sickle circulating endothelial cells abnormally express tissue factor antigen[2]. In sickle cell disease tissue factor procoagulant activity is also elevated[17]. Thrombin, interleukin -1, tumor necrosis factor and endotoxin can increase tissue factor expression. These are found to be increased in sickle cell disease[2]. The possible mechanisms that would lead to increased tissue factor expression are ischemia-reperfusion injury and increased hemolysis of type II phosphatidyl positive cells[27,32].

Patients with sickle cell disease have increased plasma levels of markers of thrombin generation in non-crisis steady state[2,32]. F1.2, TAT complexes, D - dimers, PAP complexes and fibrinopeptide A are seen in sickle cell disease in the non crises state. Decreased levels of natural anticoagulant proteins are detected in patients with sickle cell disease. Thus levels of protein

C and protein S are decreased in sickle cell disease. Reduced levels of these regulatory proteins may be a consequence of chronic consumption due to increased thrombin generation resulting from intravascular tissue factor expression and RBC prothrombinase activity. It may also be due to increased binding of protein S by sickle RBC or it may be the result of inhibition of binding of protein S to beta 2 glycoprotein 1by antiphospholipid antibodies. Anti phospholipids antibodies are also elevated in sickle cell disease[2].

It has been suggested that the circulating platelets in patients with sickle cell disease are chronically activated[2]. This also contributes to hypercoagulability in sickle cell disease. Platelet aggregation studies have found increased responses in adult patients with sickle cell disease in the non crises state[2]. This may be due to increased number of circulating active platelets or increased levels of thrombin, ADP, and epinephrine, which are platelet agonists[32].

MYELOPROLIFERATIVE DISORDERS AND THROMBOSIS

Myeloproliferative disorders are a group of disorders which are characterized by proliferation of blood cells[17,23]. The myeloproliferative disorders commonly associated with thromboembolism are essential thrombocythemia, polycythemia rubra vera and myelofibrosis with myeloid

metaplasia[23,32]. However chronic granulocytic leukemia is not associated with thromboembolism.

It has been suggested that thrombotic event in myeloproliferative disorders are platelet mediated, rather than pathologic activation of coagulation or insufficient fibrinolysis[14]. Even though the myeloproliferative disorders are accompanied by thrombocytosis, abnormal function of platelets appears to be the cause of vascular occlusion[14]. Reduced alfa adrenergic receptors in the platelets, decreased number of delta granules, decreased membrane procoagulant activity, reduced lipoxygenase activity are seen in myeloproliferative disorders, but which of these is responsible for arterial and venous thrombosis is not clear[14]. Alterations of platelet and megakaryocytic structure is seen in essential thombocythemia[14,32]. But studies have failed to relate these structural changes to an increased risk for thrombosis. Studies of platelet function have largely failed to define correlative abnormalities that could be used to define the risk for thrombosis. It was found that there is significant platelet hyperactivity in patients with essential thrombocythemia with thrombosis[14]. High blood viscosity due to high hematocrit may also be the cause of thrombosis in myeloproliferative disorders [17].

Thrombosis in myeloproliferative disorder can involve almost all levels of vascular tree including peripheral or abdominal veins, large arteries and arterioles [14,17,32]. Hepatic veins, mesenteric veins and splenic veins have

been found to have increased predisposition to thrombosis in myeloproliferative diseases [52]. Myocardial infarction, peripheral artery thrombosis, eythromelalgia, retinal artery occlusion and transient ischemic attacks are the manifestations of arterial and arteriolar thrombosis in myeloproliferative disease[14,23,52]..The laboratory diagnosis of a myeloproliferative disorder can be made when a patient has elevation of peripheral counts, splenomegaly and hypercellularity of the marrow. In some instances there may not be any significant clinical features and peripheral blood also shows only mild elevation in counts, slightly abnormal platelet morphology. In such cases, a peripheral blood or bone marow erythroid colony assay will help in correct diagnosis [17,23,52].

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired stem cell disorder characterized by complement mediated hemolysis, hypercoagulability and bone marrow failure[1]. The disease may be of autoimmune origin. It is an intrinsic erythrocyte disorder. It is the result of an aquired stem cell somatic mutation[1,17,23,51,52]. The abnormal stem cell clone produces rbc's, platelets and neutrophils that bind abnormally large amount of complement that are abnormally sensitive to complement lysis. This disorder is characterized by increased sensitivity to complement mediated lysis and is responsible for many manifestations of this disease-intravascular hemolysis with or without gross

hemoglobinuria, pancytopenia or aplasia, increased sensitivity to infections and venous thrombosis. These patients are prone to venous thrombosis peripheral veins, abdominal veins and cerebral veins are also involved[1,30,32,51,52].

PNH was first described as a distinct clinical entity in 1882. The cardinal diagnostic test, introduced in the late 1930s, is Ham's test, which is based on the increased sensitivity of PNH-affected erythrocytes to lysis by complement[10]. Deficiency of an antigen known as the membrane inhibitor of reactive lysis (CD59) is largely responsible for the hemolysis and is implicated in the tendency for patients to have thrombosis [46]. In the past two years the biochemical defect underlying PNH has been pinpointed at an early step in the biosynthesis of glycosyl phosphatidylinositol molecules — namely, the transfer of N-acetylglucosamine to phosphatidylinositol.The protein required for this step is encoded by a gene, PIG-A, that is somatically mutated in patients with PNH[46,51].

The product of the PIGA gene is required for the biosynthesis of a glycolipid anchor that attaches a class of membrane proteins known as glycosyl phosphatidyl inositol (GPI) – anchored proteins to the cell surface. The absence of GPI-anchored proteins leads to complement- mediated intravascular hemolysis, because 2 important complement regulatory proteins (CD55 and CD59) are missing from PNH cells[23]. Hemolysis in PNH occurs intravascularly. This leads to release of free hemoglobin, a potent nitric oxide

scavenger. Depletion of nitric oxide at the tissue level contributes to fatigue, esophageal spasm, thrombosis, and male erectile dysfunction[1].

Thrombosis—the leading cause of death in PNH—occurs in up to 40% of patients[1,51]. Patients with a large PNH cell population (>60% of granulocytes) seem to be at greatest risk for thrombosis. The mechanism of thrombosis in PNH is not entirely understood and is probably multifactorial; however, it must also relate to the GPI anchor protein deficiency. Nitric oxide depletion is associated with increased platelet aggregation, increased platelet adhesion, and accelerated clot formation[1]. In an attempt to repair the damage, platelets in patients with PNH undergo exocytosis of the complement attack complex, leading to formation of microvesicles lined with phosphatidylserine on their external surface. Phosphatidylserine is a potent procoagulant that is normally confined to the inner leaflet of the plasma membrane; the circulating microvesicles from PNH platelets activate coagulation and probably contribute to thrombophilia in PNH[17,23,51].

ACTIVATED PARTIAL THROMBOPLASTIN TIME AND THROMBOSIS

Activated partial thromboplastin time (aPTT or APTT) is a performance indicator measuring the efficacy of both the "intrinsic" and the common coagulation pathways [31]. Apart from detecting abnormalities in blood clotting,

it is also used to monitor the treatment effects with heparin, a major anticoagulant used in clinical practice. It is used in conjunction with the prothrombin time (PT) which measures the extrinsic pathway[10]. Prolonged APTT may indicate use of heparin, antiphopsholipid antibody or a coagulation factor deficiency[10,56]. A shortened APTT may indicate elevated coagulation factor levels[31].

As discussed previously, hypercoagulability may be due to defective naturally occurring anticoagulant mechanisms or due to increased levels of procoagulant factors. Increased levels of coagulation factors like factor VIII, IX, XI and fibrinogen is associated with thrombophilia[30,31]. These factors belong to classical intrinsic pathways of coagulation, which can be cumulatively explored by activated partial thromboplastin time[50].

Tripodi et al suggests that a shortened activated partial thromboplastin time reflect the procoagulant imbalance consequent to increased levels of coagulation factors and hence it shows an association with an increased risk of venous thromboembolism[50]. Activated partial thromboplastin time is a test which gives an overview of all coagulation factors except factor VII[32]. Hence it is a suitable test to reflect the procoagulant imbalance resulting from increased levels of single coagulation factors, which are found to be risk factor in previous studies. Tripodi et al found that patients with shortened APTT were at high risk for venous thromboembolism[50]. Previous studies also shows that

recurrence of venous thromboembolism can be predicted by doing APTT and it was found that recurrence is associated with a shortened aptt[16]. APTT is a global test responsive to plasma levels of coagulation factors of contact, intrinsic and common pathways[16,50]. Elevated levels of some of these coagulation factors have been identified as independent risk factors of venous thromboembolism. Tripodi et al's study showed that when levels of factor VIII was taken as confounding variable, the relative risk of venous thromboembolism associated with shortened APTT decreased to 2 fold, but remained statistically significant[16,50]. This indicates the high levels of factor VIII are not the only determinants of shortened APTT.

Shortened APTT have been associated with high levels of biochemical markers of thrombin generation and fibrin deposition such as prothrombin fragment 1+2, thrombin –antithrombin complex and D-dimer. It has also been found that patients with shortened APTT have a poor prognosis for thrombosis[40].

It has also been reported that lupus anticoagulant, recent thrombosis, liver disease and pregnancy affect activated partial thromboplastin time. Lupus anticoagulants are often clinically noticed by unexplained prolongation of APTT[44]. APTT is most frequently employed as the initial screening test in lupus anticoagulant[55]. Its specificity can be improved by inclusion of a mixing study with platelet free normal pooled plasma. When prolongation of APTT is due to coagulation factor deficiency, the clotting time corrects.

RESULTS

In this study, 310 cases were analysed to identify the risk factors of thrombosis. Blood samples were collected from patients presenting to various departments of PSG Institute of Medical Sciences and Research with a thrombotic tendency. Lupus anticoagulant, anticardiolipin antibody, Factor VIII assays, prothrombin time, activated partial thromboplastin time, sicklevue test, Ham's test and sucrose lysis test were done on these samples.

CLINICAL PROFILE OF THE CASES ANALYSED

124 patients had presented with proximal and distal venous thrombosis (41.3%).68 patients had recurrent abortions (22.6%). 38 cases(12.7%) had presented with ischemic heart disease. 43 cases had presented with cerebrovascular diseases. (14.3%). There were 11 cases of vasculitis (3.6%), 10 cases of arterial thrombosis and 16 cases of pulmonary thromboembolism (5.3%)

ANALYSIS OF RISK FACTORS IN DIFFERENT THROMBOTIC DISEASES

1. Venous thrombosis –proximal and distal:

124 patients had distal and proximal venous thrombosis. (41.3%) Majority of the patients had deep vein thrombosis of posterior tibial vein (29%). 8 % patients had multiple deep vein thrombosis. Multiple deep vein thrombosis was more common in intraabdominal veins. Deep vein thrombosis of axillary veins, portal veins, splenic veins and superior mesenteric veins were also studied.

Antiphospholipid antibody (LA& or ACA) was positive in 24 cases (19%). Increased factor VIII:C levels were observed in 37 patients(29.9%). 18 cases showed elevated factor VIII levels as well as antiphospholipid antibodies. Shortened APTT was present in 13 cases (10.5%). All risk factors were negative in 60 cases (54.8%).

An 18 year old female patient presented with multiple deep vein thrombosis of internal jugular vein, bilateral brachiocephalic, bilateral subclavian and partial thrombosis of inferior venacava. The patient had a prolonged prothrombin time of 27 seconds which was corrected to 12 seconds. Activated partial thromboplastin time was 31 seconds. Lupus anticoagulant was 29 seconds, IgG was 5GPLU/ml, IgM was 5.8 MPLU/ml. This patient had an elevated factor VIII levels of 200 %. Another patient with elevated factor VIII:C level was a 30 year old lady, who had developed axillary vein thrombosis. Her APTT, lupus anticoagulant assay, anticardiolipin antibodies,

sickle test, and Ham's test were within normal limits. Prothrombin time was prolonged to 25 seconds, but on correction it was 13 seconds.

A 32 year old lady had multiple deep vein thrombosis of both lower limbs. Lupus anticoagulant was positive and factor VIII was elevated in this patient. Lupus anticoagulant assay showed 67.8 seconds for screen and 51.9 seconds for confirm. Ratio was 1.3. Factor VIII level was observed to be 200%.

A 37 year old male had multiple deep vein thrombosis of lower limbs- posterior tibial vein, anterior tibial vein, saphenous vein and pulmonary embolism. All tests were normal except for a shortened APTT of 24 seconds.

A male of 54 years had come with intermittent episodes of hemoglobinuria. Ultrasound abdomen showed portal vein thrombosis. He had all tests normal, except a positive Ham's test and sucrose lysis test. That was the only case with paroxysymal nocturnal hemoglobinuria.

2. Pulmonary venous thromboembolism:

16 cases of pulmonary thromboembolism were analysed. None of them showed positive antiphospholipid antibodies. Increased factor VIII levels were observed in 8 cases (50%). Shortened APTT was observed in 3 cases (18.75%).

Increased factor VIII :C and shortened APTT was seen in 2 cases(12.5%). Sicklevue test was positive in a case (6.25%). All risk factors were negative in 6 cases (37.5%).

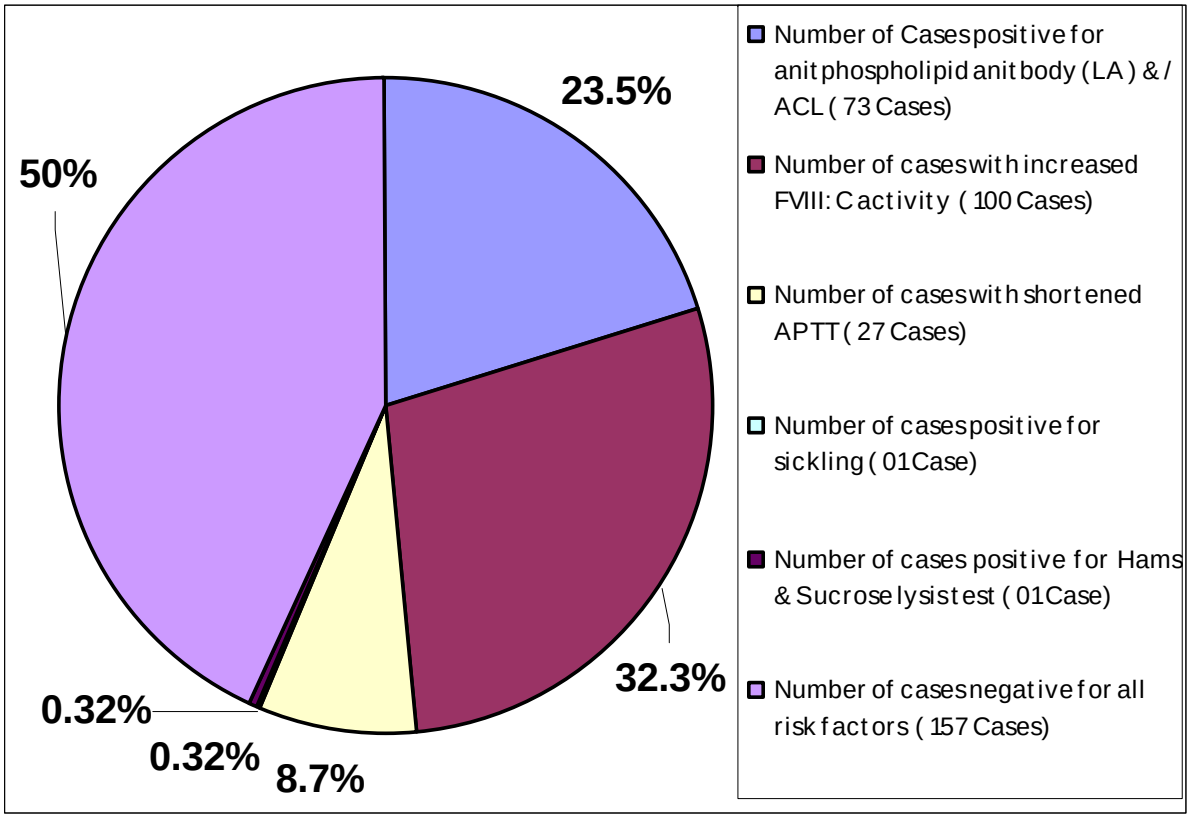
A 22 year old lady had presented with pulmonary embolism. She had moderate anemia, peripheral smear showed a mild hemolytic picture, with occasional sickle like RBC's. Sicklevue test was positive (heterozygous). Even a sickle test was done and was found to be positive. Only this case showed positivity for sicklevue test in the entire study.

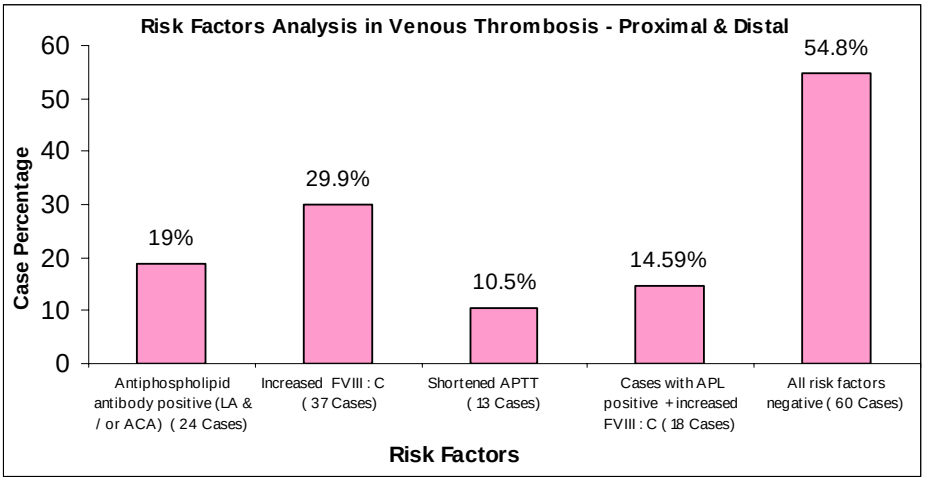
A 23 year old lady had presented with pulmonary venous thromboembolism, showed shortened APTT of 23 seconds and elevated factor VIII levels of 200%. Another patient who had shortened APTT of 22 seconds and elevated factor VIII levels of 200%, was a 63 year old male patient with pulmonary embolism.

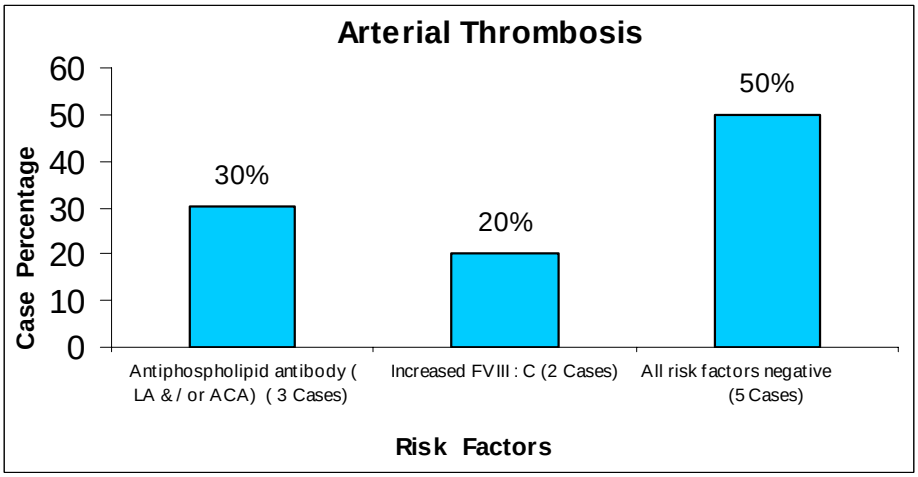
3. Arterial thrombosis:

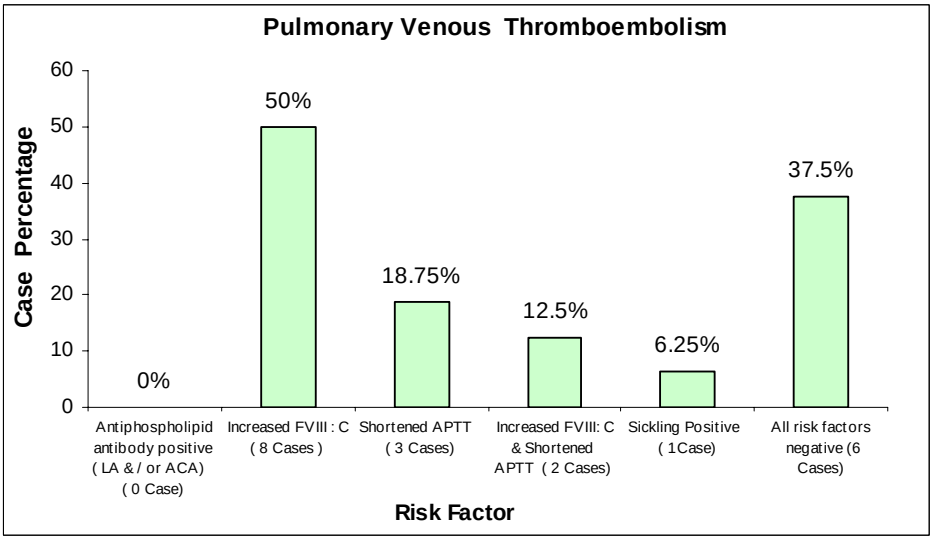
10 cases of arterial thrombosis were studied. The cases were of superior mesentric artery thrombosis, subclavian artery thrombosis and pulmonary artery thrombosis. Antiphospholipid antibodies were positive in 3 cases (30%). Increased factor VIII :C levels were seen in 2 cases (20%). All risk factors were negative in 5 cases (50%).

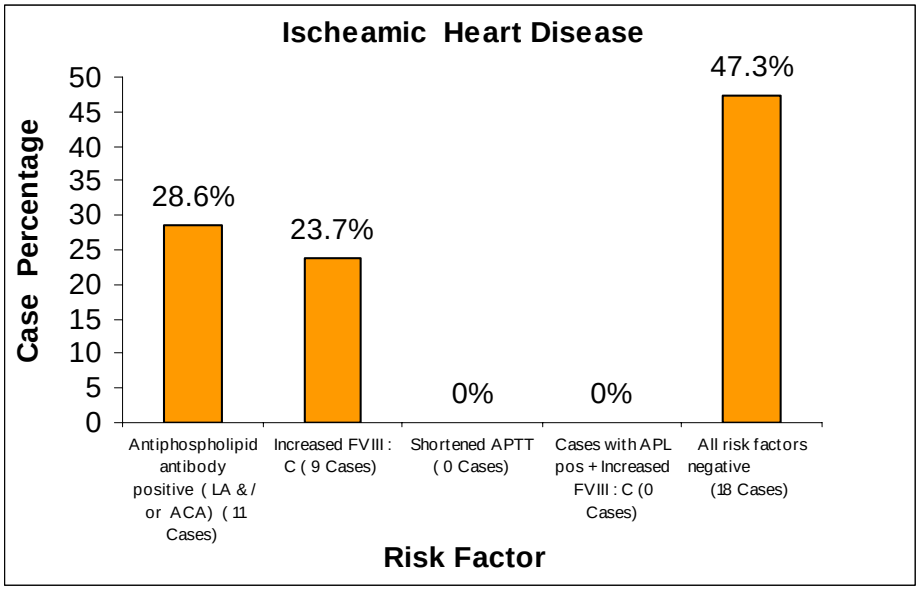
RISK FACTORS ANALYSIS

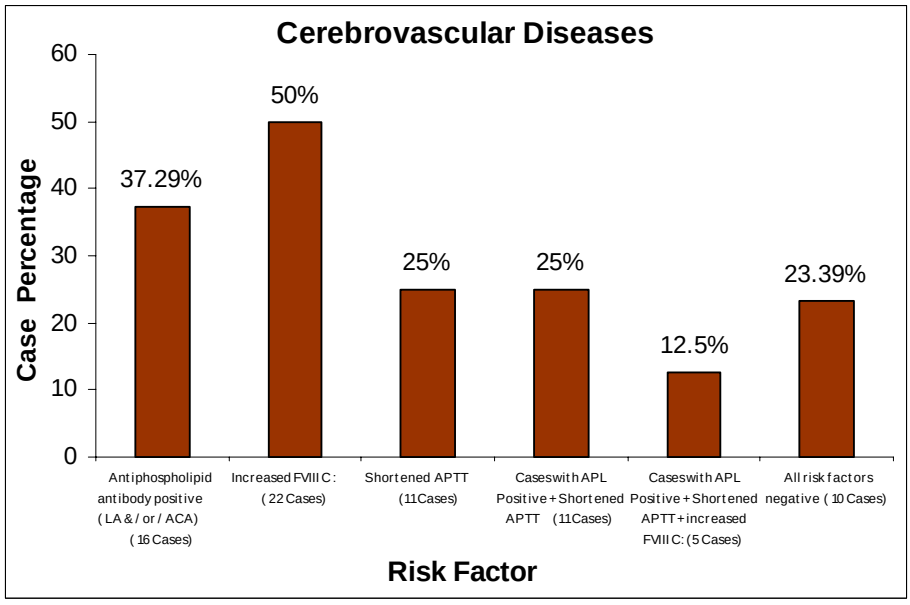


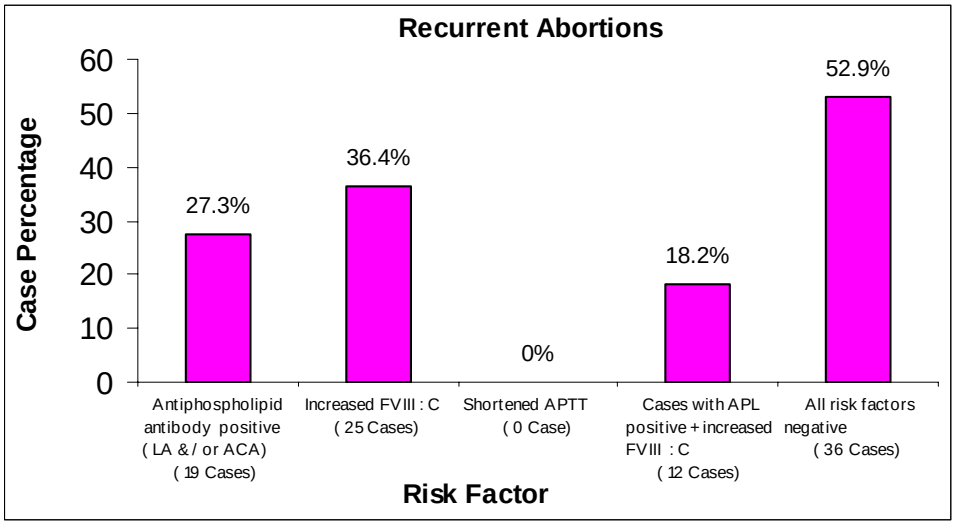

Risk Factors Analysis in Venous Thrombosis - Proximal & Distal
Case Percentage
60
50
40
30
20
10
0
19%
29.9%
10.5%
14.59%
54.8%
Antiphospholipid antibody positive (LA & / or ACA) (24 Cases)
Increased FVIII : C (37 Cases)
Shortened APTT (13 Cases)
Cases with APL positive + increased FVIII : C (18 Cases)
All risk factors negative (60 Cases)
Risk Factors

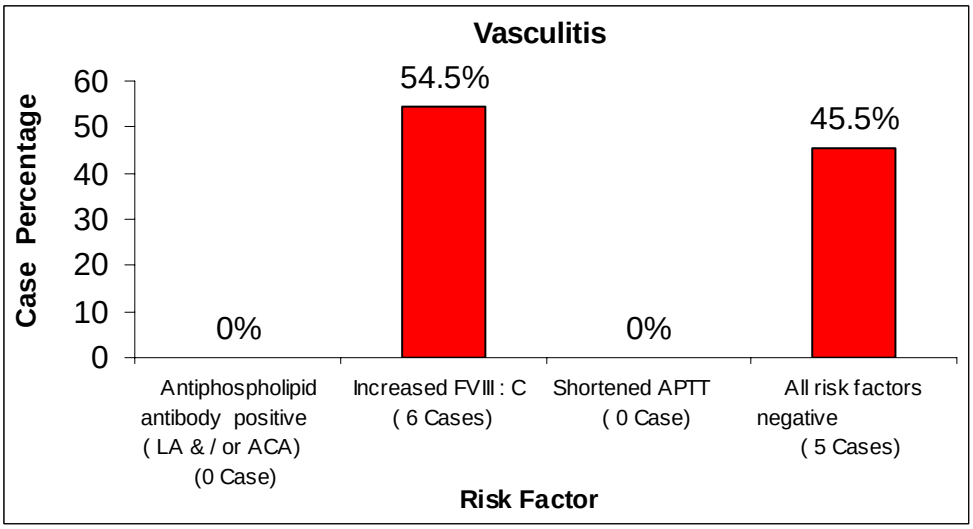

Arterial Thrombosis
Case Percentage
60
50
40
30
20
10
0
30%
20%
50%
Antiphospholipid antibody (LA & / or ACA) (3 Cases)
Increased FVIII : C (2 Cases)
All risk factors negative (5 Cases)
Risk Factors


Pulmonary Venous Thromboembolism
Case Percentage
60
50
40
30
20
10
0
0%
50%
18.75%
12.5%
6.25%
37.5%
Antiphospholipid antibody positive (LA & / or ACA) (0 Case)
Increased FVIII : C (8 Cases)
Shortened APTT (3 Cases)
Increased FVIII: C & Shortened APTT (2 Cases)
Sickling Positive (1Case)
All risk factors negative (6 Cases)
Risk Factor


Ischeamic Heart Disease
Case Percentage
50
45
40
35
30
25
20
15
10
5
0
28.6%
23.7%
0%
0%
47.3%
Antiphospholipid antibody positive (LA & / or ACA) (11 Cases)
Increased FVIII : C (9 Cases)
Shortened APTT (0 Cases)
Cases with APL pos + Increased FVIII : C (0 Cases)
All risk factors negative (18 Cases)
Risk Factor


Cerebrovascular Diseases
Case Percentage
60
50
40
30
20
10
0
37.29%
50%
25%
25%
12.5%
23.39%
Antiphospholipid antibody positive (LA & / or / ACA) (16 Cases)
Increased FVIII C : (22 Cases)
Shortened APTT (11Cases)
Cases with APL Positive + Shortened APTT (11Cases)
Cases with APL Positive + Shortened APTT + increased FVIII C: (5 Cases)
All risk factors negative (10 Cases)
Risk Factor


Recurrent Abortions
Case Percentage
60
50
40
30
20
10
0
27.3%
36.4%
0%
18.2%
52.9%
Antiphospholipid antibody positive (LA & / or ACA) (19 Cases)
Increased FVIII : C (25 Cases)
Shortened APTT (0 Case)
Cases with APL positive + increased FVIII : C (12 Cases)
All risk factors negative (36 Cases)
Risk Factor


Vasculitis
Case Percentage
60
50
40
30
20
10
0
0%
54.5%
0%
45.5%
Antiphospholipid antibody positive (LA & / or ACA) (0 Case)
Increased FVIII : C (6 Cases)
Shortened APTT (0 Case)
All risk factors negative (5 Cases)
Risk Factor

A 42 year old man had presented with ischemic bowel disease, ultrasound abdomen showed thrombosis of celiac and superior mesenteric artery. This patient had negative results for all the tests we did in the study, except for an elevated factor VIII level of 200%.

A 41 year old lady had presented with pain and edema of left upper limb and subclavian artery thrombosis, had antiphospholipid antibodies positive. Her lupus anticoagulant screen test showed a value of 68 seconds, confirm results of 70 seconds and ratio of 1.1. Her IgG value was 18 GPLU/ml and IgM value was 17 MPLU/ml. All other tests done in this study were within normal limits.

A 4 year old girl had idiopathic pulmonary hypertension and pulmonary arterial thrombus. On analysis, lupus anticoagulant and anticardiolipin antibodies were positive. Lupus anticoagulant screen value was 58.1 seconds and confirm value was 43 seconds, the ratio was 1.2. Anticardiolipin assay showed IgG levels of 18.2 GPLU/ml and IgM levels of 22.1 MPLU/ml. Factor VIII level was normal. This patient was the youngest patient in the study.

4. Ischemic heart disease:

38 cases of ischemic heart disease were analysed in this study. There were cases of myocardial infarction and acute coronary syndrome. Antiphospholipid antibodies were positive in 11 cases (28.6%). Increased factor VIII:C was seen in 9 patients (23.7%). All risk factors were negative in rest of the 18 cases.(71.4%)

A 27 year old male patient presented with acute coronary syndrome. His prothrombin time was prolonged, but corrected prothrombin time was normal. APTT was 30 seconds. Lupus anticoagulant assay screen test was 60.1 seconds and confirm test was 56 seconds and the ratio was 1.1, which indicates a positive result for lupus anticoagulant assay. Anticardiolipin antibodies were also positive in this patient. IgG level was 20 GPLU/ml and IgM level was 22.1 MPLU/ml. Factor VIII level was in the upper limit of normal, i.e 149%.

A 25 year old male had presented with myocardial infarction. All the investigations done in this study were normal except for increased factor VIII level of 200%.

5. Cerebrovascular Diseases

There were 43 patients with cerebrovascular diseases like superior sagittal sinus thrombosis, as well venous and arterial infarcts in the brain

parenchyma. 16 patients (37.2%) had antiphospholipid antibodies (either lupus anticoagulant or anticardiolipin antibodies or both.). Increased factor VIII levels were seen in 22 cases (50%). Shortened APTT was seen in 11 cases. All these cases had antiphospholipid antibodies positive. Out of this, 5 patients (12.5%) had increased factor VIII levels as well. All risk factors were negative in 10 patients (23.3%).

A 63 Year old female presented with superior sagittal sinus thrombosis. Her activated partial thromboplastin time was shortened i.e 24 seconds, and antiphospholipid antibodies were positive. Lupus anticoagulant screen test was 72.6 seconds and confirm time was 74.6 seconds. The ratio was 1.1. Anticardiolipin antibody assay showed IgG to be 28.8 GPLU/ml and IgM value of 30.8 MPLU/ml. Factor VIII level was within normal limits.

A 67 year old male had infarct in the left corona radiata and lacunar infarcts in thalamus. His APTT was shortened with a value of 23 seconds. Lupus anticoagulant screen time was 62 seconds, and confirm time was 50 seconds, the ratio was 1.2. which indicates a positive result for lupus anticoagulant. Anticardiolipin antibodies were positive. IgG level was 23 GPLU/ml and IgM level was 23 MPLU/ml. Factor VIII was also elevated in this patient with a factor VIII level of 200%.

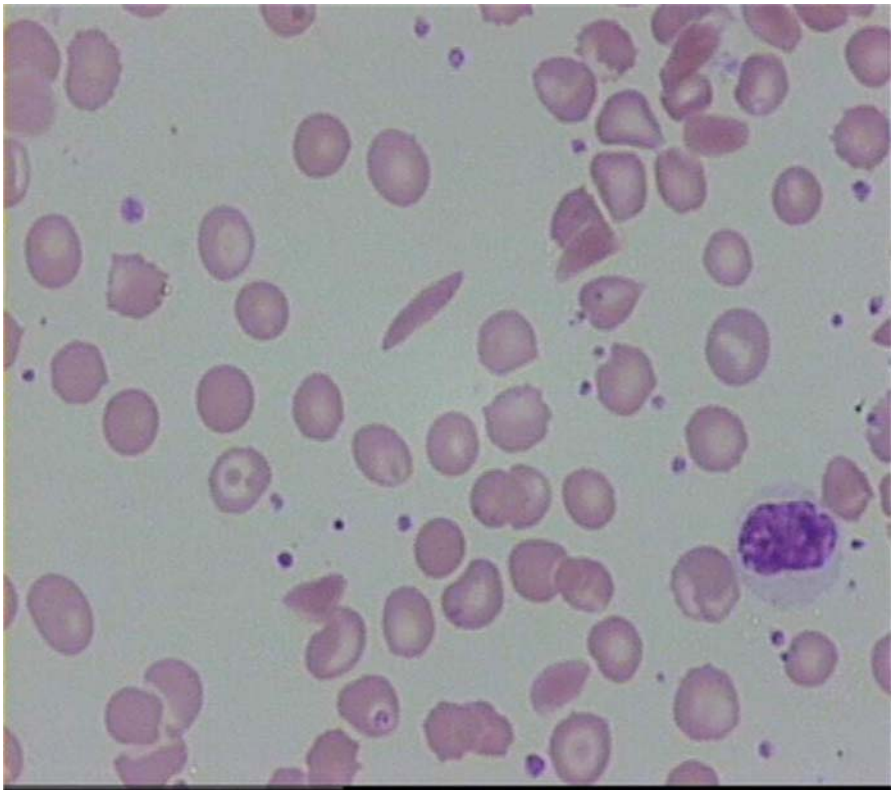
6. Recurrent abortions

Women referred to the obstetrics department with a history of early, recurrent abortion (at least three pregnancy losses before 13 weeks of gestation) were eligible to be included in this study. Exclusion criteria were endocrine, immunological or anatomical causes of embryo demise. 68 cases of recurrent abortion were included in this study. 19 cases (27.3%) were positive for either lupus anticoagulant or anticardiolipin antibody or both. Increased factor VIII levels were seen in 25 cases (36.4%). 12 cases (18%) showed positivity for antiphospholipid antibodies as well as elevated factor VIII level. All risk factors were negative in 36 cases (52.9%).

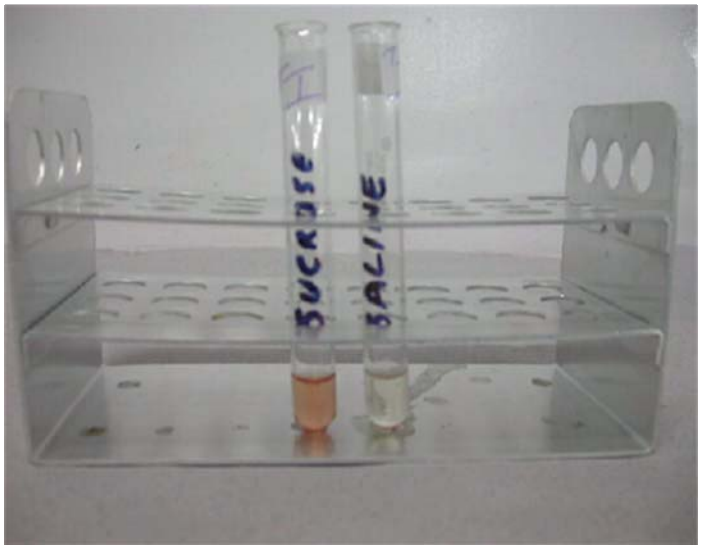
7. VASCULITIS

11 cases of vasculitis were included in the study. 6 patients had elevated factor VIII levels (54.5%). All risk factors were negative in rest of the 5 cases (45.5%)

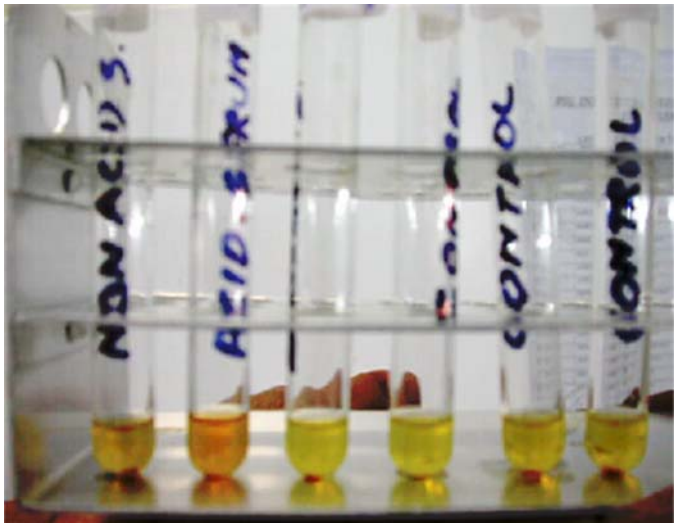
SICKLE CELL ANEMIA



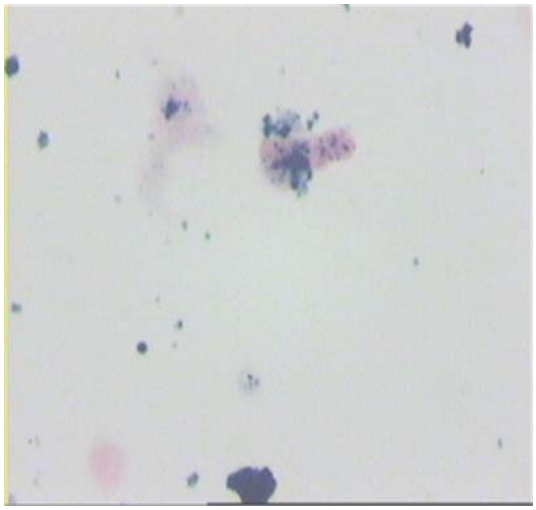
SUCROSE LYSIS TEST (Positive)



HAM'S TEST (Positive)



URINE DEPOSIT - PNH



DISCUSSION

The epidemiology of venous thromboembolism (VTE) in the community has important implications for the prevention and management of this disease. In order to improve survival, avoid recurrence and prevent complications, the occurrence of VTE must be reduced. To reduce the incidence of VTE, persons at risk for thromboembolism must first be identified. Independent risk factors for VTE include patient age, surgery, trauma, hospital confinement, active malignancies, neurological diseases and procedures like central vein catheterization. Recent family based studies indicate that VTE is highly heritable and follows a complex mode of inheritance involving environmental interactions[18]. Inherited reductions in plasma natural anticoagulants – antithrombin, protein C, protein S -have long been recognized as uncommon but potent risk factors for VTE. More recent discoveries of impaired down regulation of the procoagulant system like Activated Protein C resistance, Factor V Leiden[15] and increased plasma concentrations of procoagulant factors such as factors I, II, VIII, IX, and XI[19] have added a new dimension to the list of inherited or acquired disorders predisposing to thrombosis[19,35].

The identification of risk factors for thrombosis is a controversial topic. However, such studies should help identify a vulnerable population and help

target prophylaxis to those who benefit the most and ultimately reduce the occurrence of VTE. With the above objectives in mind, 310 patients with symptomatic venous or arterial thrombosis were evaluated to identify the possible etiology of the thrombosis. Patients were classified according to their underlying disease syndrome. The major categories included patients having proximal or distal venous thrombosis (40.0%). 68 patients who attended the obstetrics out patient department with history of recurrent abortions were included in this study (21.9%). Other thrombotic events included CNS arterial thrombosis (13.9%), coronary artery disease and myocardial infarctions (12.3%), pulmonary embolism (5.2%) and vasculitis (3.5%) and arterial thrombosis (3.2%).

The investigations done included coagulation tests (prothrombin time, activated partial thromboplastin time), factor VIII:C (one-stage APTT based), lupus anticoagulant (dRVVT based), anticardiolipin antibodies, acidified serum test and sickling test.

Increased factor VIII:C was the commonest risk factor identified (32.3%), followed by antiphospholipid antibody (23.5%) and shortened APTT (8.7%). No risk factors were identified in 50.6% of the cases. 15.5% of the cases had multiple risk factors positive – positive APL with increased factor VIII:C, increased factor VIII:C and shortened APTT, and in 12.5% of the patients who presented with cerebrovascular symptoms had a combination of

APL positivity together with increased factor VIII:C levels and shortened APTT.

There was one case each of sickle cell anemia and paroxysmal nocturnal hemoglobinuria who presented with pulmonary embolism and portal vein thrombosis respectively.

ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibody syndrome is an important clinical entity with a strong tendency for thrombosis accompanied by high morbidity and mortality. The diagnosis is made when arterial or venous thrombosis or recurrent pregnancy failure occurs in a patient with a persistently positive laboratory test result for an antiphospholipid antibody – lupus anticoagulant &/or anticardiolipin antibody.

In the study done, 23.5% of patients who presented with venous thrombosis were positive for antiphospholipid antibodies – lupus anticoagulant &/or anticardiolipin antibodies. This incidence is similar to that of Steven et al who found the occurrence rate of antiphospholipid antibodies in venous thrombosis to be 24%[7]. Paul E. Love and colleagues had similar results (45%)[25]. Petri et al and Fort et al had 11% and 31% respectively of patients with positive anticardiolipin antibodies[25]. Bhattacharya et al had 40%

patients with positive antiphospholipid antibodies associated with thrombosis[4]. The study done by Pengo et al showed LA and /or anticardiolipin antibodies positive in 34% of patients with thrombosis[38].

Literature shows that antiphospholipid antibodies are positive in young patients <45 years[5,7,25]. Most of the studies showed antiphospholipid antibodies in age group between 20- 55 years. In our study, the youngest patient was a 4 year old girl with pulmonary arterial thrombosis.

This study has shown positivity for lupus anticoagulant and anticardiolipin antibodies in other clinical situations like arterial thrombosis, ischaemic heart disease and in patients with recurrent abortions. 34% of the patients positive with antiphospholipid antibodies had arterial thrombosis. The diagnosis of Lupus anticoagulant followed the criteria set by the Subcommittee for the Standardization of Lupus Anticoagulants of the International Society of Thrombosis and Haemostasis[6].

Jerrold .S.Levine and colleagues had 27% of patients with LA and or anticardiolipin antibodies[3,7,44]. Bhattacharya et al's study showed 40% patients with lupus anticoagulant with arterial thrombosis[4]. Paul E Love had 22% cases with arterial thrombosis and positive lupus anticoagulant[25].

Antiphospholipid antibody syndrome has emerged as the most important treatable cause of recurrent miscarriage, early onset preeclampsia and of intrauterine growth retardation. 27.3% of our patients with recurrent pregnancy loss were positive for either lupus anticoagulant or anticardiolipin antibody or both. Bhattacharya et al found 22% of patients had recurrent fetal loss associated with antiphospholipid antibodies[4]. Paul E Love has reported eight series of retrospective analyses of fetal loss in patients with antiphospholipid antibody[25]. In these studies, the patients who had one or more fetal losses ranged from 13 to 58%. Jerrold et al had 20% of patients with antiphospholipid antibody presenting with recurrent fetal loss[44].

Anticardiolipin antibodies are not such strong risk factors for thrombosis as lupus anticoagulants. Separate analysis of different types of thrombosis show that ACA are associated more with cerebral stroke and myocardial infarction, and not with DVT[27]. In the present study also, it was observed that 28.6% and 37.2% respectively of cases of ischaemic heart disease and cerebrovascular disease were associated with positive anticardiolipin antibodies. Only 19.6% of cases with venous thrombosis were positive for anticardiolipin antibodies.

This study did not reveal any statistically significant difference in the APL positivity for the following groups of patients – patients with IHD and recurrent abortions, patients with venous thrombosis and cerebrovascular

disease and patients with PVTE and vasculitis. In fact, there were no positive APL case in this last group of PVTE and vasculitis.

However, the investigation brought out a significant difference in the contribution of APL as a risk factor for venous thrombosis when compared to pulmonary thrombo-embolism ($p=<0.05$). The APL positivity seen in venous thrombosis was more than what was observed in PVTE. Similarly, there is a statistically significant difference in the contribution of APL for PVTE and IHD ($p=0.003$) as well.

According to Ling Zhang et al[55]. LA testing necessitates investigation by at least two or more methods before it is considered negative. In the present study, it was observed that 30 patients were positive for only LA, 43 patients were positive for ACA and 22 patients showed positivity for both LA and ACA. However there are 237 patients in this study who presented with thrombosis and were negative for both tests. Laboratory testing for LAs is not standardized, and various combinations of screening, confirmatory and integrated test systems can be used. The conventional tests that were used – the APTT and the dRVVT, may be insensitive to the presence of LAs, necessitating the use of multiple assays before reporting a patient sample as negative for LA.

The APTT is considered to be a readily available and inexpensive screening test for LAs. Zhang et al found only 13 (46.4%) of 28 samples testing positive for LAs with a prolonged PTT-LA result [55]. The present study did not show prolonged APTT in any of the cases that tested positive for LA. From the above findings, the APTT cannot be considered an optimal screening test for Lupus anticoagulants.

ELEVATED FACTOR VIII:C

Recently, a growing literature has suggested that elevated levels of factor VIII:C may represent a risk factor for VTE [22,37]. Coagulation is triggered during the onset of myocardial infarction, resulting in vascular occlusion. A number of studies have examined the association of cardiovascular disease with coagulation factors (fibrinogen, factor VII, factor VIII, platelet aggregability) and fibrinolytic factors (tissue plasminogen activator, plasminogen activator inhibitor-1, Lp(a) lipoprotein, plasminogen activity). Of these, a significant association was seen between increased fibrinogen levels and ischemic heart disease. Relatively less is known about the relation between factor VIII and cardiovascular disease risk.

In the present study, out of 124 patients who presented with proximal and distal venous thrombosis, 29.9% patients had increased factor VIII levels,

14.5% had increased factor VIII levels as well as antiphospholipid antibodies. Rodereik et al[5], and Kyre et al[22] had similar results (25%, 27% and 29%). O'Donelle's study also showed 26% cases with elevated factor VIII[37]. Kraaijenhagen et al found high levels of factor VIII in 57% of patients with recurrent venous thromboembolism[21].

This study shows a significant association between elevated factor VIII:C levels and myocardial disease (23.7%). The presence of elevated factor VIII:C levels in PVTE were far more significant than the levels in IHD and arterial thrombosis.

A study done by Benchimol et al examined the predictive value of haemostatic factors for sudden death in patients with stable angina pectoris[3]. Factor VIII coagulant was found to be a univariate predictor of sudden death in this population. In the Northwick Park heart study, mean factor VIII:C concentrations were significantly higher in patients who died of ischaemic heart disease compared with survivors[34]. Of 16 cases of pulmonary embolism screened in this study, 50% had elevated factor VIII levels. Kamphuisen et al report 61% of patients with pulmonary embolism having elevated factor VIII levels[39]. Well's et al's study also shows a high number of 51% of patients with pulmonary embolism having elevated factor VIII levels[54]. In their study the authors have taken values more than 200 IU/dl as being significant.

Factor VIII is an important acute phase reactant, hence the elevated levels should be considered significant only if they continue to be abnormal several weeks after the thrombotic event. Well's et al also suggests that age and gender should be considered when analyzing factor VIII levels[54].

A number of inherited and acquired coagulation abnormalities are associated with dural sinus thrombosis. One of the most common and well documented of these is the factor V Leiden mutation G1691A seen in 15 to 20% of cases[26]. In the present study, there was a significant percentage of patients (50%) who presented with superior sagittal sinus thrombosis with elevated factor VIII:C levels as well as shortened activated partial thromboplastin time.

Known causes or associations of recurrent miscarriage are endocrine, immunological, anatomical and genetic causes, although about 50% of cases are classifiable as idiopathic. In recent years, attention has to be drawn to the possible association of both early and late pregnancy loss with either inherited or acquired thrombophilic defects predisposing to the development of DVT, namely, FV R506Q (F V Leiden), and prothrombin gene G20210A mutations, hyperhomocysteinaemia and antiphospholipid antibodies.

The present study revealed a high association between elevated factor VIII: C levels and patients with early recurrent abortions (36.4% of patients).

This finding suggests a possible association between this thrombophilic condition and early reproductive failure. This data is in concurrence with the study by Marietta et al[36] in which 25.5% of the patients studied had Factor VIII:C levels exceeding the 90th centile of the control population. Dossenbagh and colleagues also found 25% of patients with elevated factor VIII levels presenting with recurrent abortions[13].

It could be hypothesized that hypercoagulable states can lead to some vascular impairment in the first phases of embryo implantation. In physiological conditions, the F VIII:C concentration rises as pregnancy advances. A gradual dose-response relationship between F VIII levels and the risk of thrombosis has been observed. Therefore, it can be argued that in predisposed women even a small increase in already elevated F VIII:C levels can attain a hypothetical threshold required for triggering the thrombotic event.

Many acquired variables, above all acute phase reactions may increase F VIII:C activity. In order to avoid inflammatory response as a confounding factor we should have ideally included measurement of C reactive protein (CRP) levels, an established acute-phase marker in all our patients. Given the variability of F VIII:C levels due to several pathophysiologic factors, however, it remains to be established whether or not FVIII:C levels should be included in screening for thrombophilia in such patients.

SHORTENED ACTIVATED PARTIAL THROMBOPLASTIN TIME

The activated partial thromboplastin time is a simple coagulation test that has been in routine laboratory use for decades. An APTT reagent contains suitable concentrations of phospholipid and activator and detects coagulopathies of the intrinsic and/or common pathway. If preanalytical variables can be excluded, then a shortened APTT could reflect in vivo activation and potentially be a marker for thrombosis.

The phenomenon of an abnormal resistance to the anticoagulant activity of activated protein C (APC) was first described by Dahlback in 1993[11]. Dahlback showed evidence for an increased prevalence of APC resistance among patients with familial venous thromboembolism. In his study, DVT patients with abnormal APC resistance had shorter APTTs than those with normal responses to APC. This was just prior to the Factor V Leiden being identified (Bertina et al, 1994). Until recently, it was unclear whether a link existed between VTE and the phenotype of abnormal APC resistance independent of F V Leiden.

The results of investigations associated with the Leiden Thrombophilia study[53] have shown that non-FV Leiden APC resistance, as quantified in an APTT-based screening test, is an independent marker for VTE. Lowe et al[24] in

attempting to define a preoperative hypercoagulable state that may predict postoperative DVT in surgical hip replacement patients assessed the relevance of 29 hemostatic factors in 480 patients. Only the shortened APTT and the APC resistance ratio showed a statistically significant association with the development of postoperative DVT.

McKenna et al[31] found that 23% developed VTE in a prospective study of 100 medical and surgical patients with shortened APTTs. In the control population of medical and surgical patients during the study period, only 2.2% developed VTE. There was a correlation between the degree of shortening of the APTT and the incidence of VTE in this study.

In the present study, 8.7% of cases had shortened APTT. Of these 27 patients, 16 had venous thrombosis including pulmonary venous thrombosis. Two of these patients had in addition elevated factor VIII levels. Out of a total of 109 patients with elevated factor VIII:C levels, 27 (25%) had shortened activated partial thromboplastin times. 11 patients who had cerebrovascular diseases had multiple risk factors– shortened APTT and positive antiphospholipid antibody and 5 of these patients, in addition had increased factor VIII levels. This indicates that the cause for the low APTT is more complex than just increased FVIII levels.

Tripodi et al have reported shortened APTT as a risk factor for venous thromboembolism with 19% cases with shortened APTT[50]. This observation is supported by the findings of Boekel et al[47] and Reddy[40]. In Tripodi's study 83 % of the patients had deep vein thrombosis, 12% with isolated pulmonary embolism and 5% with cerebrovascular diseases. He also observed an increased factor VIII level with shortened APTT in 27 % of the cases.

It was observed that there was a statistically significant difference in the presence of shortened APTT in cerebrovascular disease compared to IHD. The literature survey revealed only a single study of a case of fatal dural sinus thrombosis associated with a shortened APTT. In this case, factor VIII levels were not measured so the basis for the short APTT was unknown[8].

SICKLE CELL ANEMIA

Only one of the patients in this study tested positive for sickle cell anemia. This patient presented with pulmonary embolism. Stein PD and colleagues in a similar study observed deep vein thrombosis to be the commonest thrombotic event in sickle cell disease. Austin et al observed 6% of cases with sickle cell disease and venous thrombosis. This study however, was limited to a black population[45].

PAROXYSYMAL NOCTURNAL HEMOGLOBINURIA

A case of paroxysymal nocturnal hemoglobinuria presented with portal vein thrombosis in our study". Araten DJ[1] and colleagues found 14% of PNH patients with venous thrombosis, mostly intraabdominal venous thrombosis. Valla D et al [51] and Ziakas et al [58] also have reported a single case of portal vein thrombosis in paroxysymal nocturnal hemoglobinuria.

The present study was limited to only acquired risk factors since at the moment, facilities for genetic studies are not available at our institution. In addition, the confirmation of antiphospholipid antibodies as risk factors can be done only if repeat testing after a period of 6 weeks is performed. Only 15 patients (4.8%) came back for repeat testing. However, their results were encouraging since all of them continued to be positive for Lupus Anticoagulant and Anticardiolipin antibodies.

Therefore, although this study had significant observations and correlations of risk factors for thrombosis, more detailed studies will have to be performed

SUMMARY AND CONCLUSIONS

The present study was aimed at examining risk factors involved in the pathogenesis of thrombosis in the Indian population. Review of literature has shown that most of the epidemiology studies to date with reference to thrombophilias, are confined to data derived from the European population.

This study was done on 310 patients who presented with different thrombotic disorders. While the clinical utility of diagnostic testing for an inherited or acquired thrombophilia remains controversial, studies such as these hold the potential of further stratifying individual patients into high- and low-risk for incident and recurrent VTE. This in turn will help target prophylaxis to those who benefit the most and ultimately reduce the occurrence of venous thromboembolism.

The commonest risk factor identified was increased Factor VIII:C levels, followed by antiphospholipid antibody and shortened activated partial thromboplastin time. Two hemolytic anemias – one genetic (sickle cell anemia) and the other an acquired membrane disorder (paroxysmal nocturnal hemoglobinuria) were also found to be associated with a tendency to thrombosis. 50% of the patients in this study who presented with thrombosis had no identifiable risk factors.

The presence of antiphospholipid antibodies was found to be linked with ischemic heart disease - myocardial infarction and acute coronary syndromes and venous and arterial thrombosis. There was also a very significant association between the presence of antiphospholipid antibodies – lupus anticoagulant and anticardiolipin antibodies with recurrent abortions. Antiphospholipid syndrome has emerged as the most important treatable cause of recurrent miscarriage and of intrauterine growth restriction. Though we did include a study of 20 control samples in our study in addition to the control samples which were run simultaneously with the test samples, there is a need to study baseline levels of anticardiolipin antibodies in a larger number of the Indian population, to determine at what levels these patients should be treated.

There is abundant evidence that thrombosis is involved in the acute presentation of coronary, cerebrovascular, and peripheral vascular diseases. Relatively less is known about the relation between factor VIII and cardiovascular disease risk. This study showed a significant association between elevated FVIII:C levels and almost all of the thrombotic disorders with the highest presence of elevated factor VIII in pulmonary thromboembolism.

This study has also demonstrated a significantly higher frequency of subjects with elevated FVIII:C levels in women with early, recurrent abortions.

A shortened APTT has been reported as a risk factor for thrombosis. There was a correlation between the shortening of the APTT and the incidence of venous thromboembolism in this study. In addition, it was also observed that nearly 25% of the patients with elevated FVIII:C levels had shortened APTTs.

The study of antiphospholipid antibodies in this study was done with two tests – the DRVVT test for LA and the ELISA test for ACA. It is important to note that for the diagnosis of antiphospholipid antibody syndrome, patients who test LA and ACA negative by the above methods must also be tested with other assays for lupus anticoagulant. This study also showed that the APTT is not an optimal screening test for detecting lupus anticoagulants.

LA may be positive in 1-5% of healthy adults, but none of the control samples in this study were positive for LA.

It must be stressed that each laboratory should define its own reference ranges for all investigations. This study also underlines this fact of the importance of locally derived reference ranges.

This study demonstrates that the following categories of patients must be tested for risk factors for thrombosis - patients with recurrent idiopathic thrombosis, deep vein thrombosis, thrombosis in unusual sites, association of arterial and venous thrombosis, recurrent abortions, early age of onset, and patients with a family history of venous thromboembolism. The investigations must include both acquired as well as genetic factors contributing to thrombosis. It is important to stratify patients according to their risk factors in order to target primary and secondary prophylaxis to the patients who would benefit the most.

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KEY TO MASTER CHART

ACL - ANTI CARDIOLIPIN ANTIBODY

Ant - ANTERIOR

C - CONTROL

CVA - CEREBROVASCULAR ACCIDENT

DVT - DEEP VEIN THROMBOSIS

IHD - ISCHEMIC HEART DISEASE

LA - LUPUS ANTI COAGULANT

Lt - LEFT

Post - POSTERIOR

R - RATIO

Rt - RIGHT

SMA - SUPERIOR MESENTRIC ARTERY

SMV - SUPERIOR MESENTRIC VEIN

SSS - SUPERIOR SAGITTAL SINUS

VTE - VENOUS THROMBOEMBOLISM